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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
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Application for Research Grant
(Use extra pages as needed)

Date: Dec. 23, 1974

1. Principal Investigator (give title and degrees):
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3. Department(s) where research will be done or collaboration provided:

Division of Microbiology, Department of Laboratories.

4. Short title of study: The Influence of Cigarette Smoke on Intrapulmonary Inactivation of Inhaled Bacteria by Alveolar Macrophages.

5. Proposed starting date: July 1, 1975

6. Estimated time to complete: 3 years

7. Brief description of specific research aims: The proposed project is an in vivo investigation of acute and extended exposure to whole cigarette smoke and its vapor phase on the ability of alveolar macrophages to ingest and destroy inhaled bacteria. Methods are used which permit an evaluation of:

(1) The effect of cigarette smoke on lung clearance of bacteria as related to the rate of ingestion of inhaled bacteria by pulmonary macrophages.

(2) In vitro methods for studying phagocyte-bacterium interactions will be used to determine the effect of in vivo exposure to cigarette smoke on (a) chemotactic responsiveness to a bacterial stimulus, (b) adherence of bacteria to the surfaces of macrophages, and (c) the rate at which phagocytized bacteria are destroyed.

(3) Studies will be performed to delineate the effect of cigarette smoke on the contributions of bronchial washings and of pulmonary secretions (Secretory IgA) to the antibacterial activity of alveolar macrophages.

(4) Studies are also presented to assess the influence of smoke inhalation on the metabolic activities of alveolar macrophages in phagocytosis and destruction of bacteria.

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8. Brief statement of working hypothesis:

The current grant proposal is based on evidence which demonstrates the following: (a) alveolar macrophages play a key role in intrapulmonary inactivation of inhaled bacteria, (b) bronchopulmonary secretions (IgA) and bronchial washings contribute to pulmonary defense and may act as mediators of effective alveolar macrophage function. (c) studies aimed at providing a meaningful evaluation of the effect of tobacco smoke on pulmonary defense mechanisms are best performed under in vivo conditions of smoke exposure. The investigations proposed herein emanate from observations made in this laboratory which indicate that acute and extended (15 days) exposures to cigarette smoke cause an impairment of lung clearance of bacteria that is reversible within hours after the cessation of smoke exposure. In addition, smoke inhalation provokes a selective mobilization of alveolar macrophages without interfering with the mobilization of macrophages in response to the inhalation of bacteria. Similarly, in vivo smoke exposure results in a reversible suppression of the phagocytic ability of alveolar macrophages. Finally, the importance of macrophage mobilization in protecting the lung against cigarette smoke was also demonstrated. These findings indicate the need for further investigations and for studies of the influence of cigarette smoke as relates to the immunological and biochemical properties of alveolar macrophages in the phagocytosis of bacteria.

See pages 16 - 33 for a detailed report of the Background Material and Supporting Data that serve as the basis for the studies proposed in this grant.

9. Details of experimental design and procedures (append extra pages as necessary)

See pages 2A (1-15)

Table of Contents

9. Experimental Design: Study of the Influence of Cigarette Smoke on Disposal of Inhaled Bacteria by the Respiratory Tract.

Reference Response System: Clearance of *Staphylococcus aureus* by the Respiratory Tract

Sites of Respiratory Tract clearance to be studied.	Trachea and Major Bronchi (Tracheal Clearance Studies)	Lungs (Lung Clearance Studies)
Major Respiratory Tract Defense System Operative	Mucociliary apparatus	Alveolar Macrophage System
Particle Size of Bacterial Aerosol Required for Proposed Studies	5.0μ to 0.5μ	
Aerosol Generating unit to be used	DeVilbiss No. 40 Nebulizer	

Alveolar Macrophage System

Parameters of Alveolar Macrophage Activity to be studied	Mobilization	Phagocytosis
	(a) Chemotaxis (b) Migration rates (c) Cell adhesiveness	(a) Attachment of bacteria to macrophage surface (b) Ingestion of bacteria by macrophages (c) Intracellular destruction of ingested bacteria by macrophages

Areas of Comparative Assays by Laboratory

Immunologic Medicators of the Phagocytic Act	Metabolic Indices of the Phagocytic Act	Metabolic Indices of Bactericidal Activity
(a) Serum factors (b) Bronchial washings (c) Pulmonary secretions (SIgA)	(a) Respiration (b) Glucose metabolism (c) ATPase activity	(a) Catalase and peroxidase activity and H_2O_2 production (b) Hydrolytic enzyme activity

Experimental Procedures:

All studies will be performed under in vivo conditions of smoke-exposure (See Methods of Procedure Page 2A-8)

A. Observations of Tracheal and Lung Clearance: The following specific information is sought from these studies: (a) the effect of extended exposure to whole cigarette smoke and its gas-phase on tracheal and lung clearance of bacterial deposits and (b) the reversibility of any adverse change in clearance rates attributable to smoke-exposure. For this purpose, experimental and control mice will be first exposed to cigarette smoke and a secondary air flow, respectively, and then challenged with aerosols of S. aureus for 30 minutes. Immediately following aerosol exposure (0 time) some of the smoke-exposed and control animals will be sacrificed to determine the number of bacteria initially deposited in their trachea and lungs. The remainder will be killed 15 min, 30 min, 1 hr, 2 hrs and 4 hrs after bacterial challenge to ascertain the number of viable bacteria residing in the trachea and lungs of smoke-exposed and control mice. From these data, it is possible to calculate the rate at which bacteria are cleared from the trachea and lungs. These studies will be performed in mice exposed daily for 1.0 hour to cigarette smoke over a 3 week period. Tracheal and lung clearance rates will be measured immediately following and 24 to 48 hrs after exposure to cigarette smoke for 1, 5 and 15 days. (See Methods of Procedure Pgs. 2A(8-9)-).

B. Alveolar Macrophage Activity: These studies will be performed under the same conditions of smoke-exposure used to assess the effect of extended exposure to whole and gas-phase cigarette smoke on tracheal and lung clearance. In this regard, mice and/or rabbits will be exposed daily for 1.0 hr to cigarette smoke over a 3 week period and alveolar macrophage activity will be assessed immediately following and 24-48 hrs after daily exposures to smoke for 1, 5 and 15 days, respectively.

(1) In Vivo Phagocytosis and Intracellular Killing Activity: Studies will be performed to determine the effect of extended exposure to whole and gas-phase cigarette smoke on the antibacterial activity of alveolar macrophages in the intact and functioning lung. For this purpose experimental and control mice will be first exposed to cigarette smoke and a secondary air flow, respectively, and then challenged with staphylococcal aerosols for 30 minutes. Immediately after bacterial challenge (0 time) and at hourly intervals during a 4 hour post-aerosol exposure period, alveolar macrophage harvests obtained from smoke-exposed and control animals will be processed as follows to obtain data on phagocytosis and intracellular killing: bacterial counts will be obtained from an aliquot of the total lung washout and the remainder will be separated by differential centrifugation at 1500 rpm for 15 min into a supernatant fraction containing free bacteria and a cellular fraction laden with alveolar macrophages and phagocytized bacteria. The number of viable bacteria present in each fraction will be determined by a standard pour plate technique. The decrease in the number of viable bacteria present in the total lung washout, as a function of time will be used as an index of the clearance rate of the lung sample obtained by the lavage technique. Similarly, a decrease in the viable counts of the supernatant and cellular fractions as a function of time, will be used as a measure of the ingestion and intracellular killing of bacteria by alveolar macrophages. (See Lung Harvest Method p. 2A-9).

In a second series of studies groups of smoke-exposed and control mice will be exposed to staphylococcal aerosols as outlined above. At 0 time, 1, 2 and 4 hrs after infection, groups of control and smoke-exposed animals will be sacrificed and the lungs of each mouse exposed and perfused with 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 via polyvinyl chloride catheter threaded into the distal tracheal segment. The fixed lung will be embedded in paraffin and sections

cut at 4 to 5 μ. The sections will be stained with Brown and Brenn tissue stain for bacteria (Preece, A. Manual for histologic techniques. 1972. Little, Brown & Co. Boston, p 320) and scanned for staphylococci with a Leitz Orthoplan microscope. The intra and extracellular location of 500 consecutive bacteria will be determined and used as a measurement of the proportion of inhaled bacteria phagocytized by control and smoke-exposed mice (Goldstein et al, J.Clin. Invest., 54:519, 1974). These studies will be performed under the same conditions of smoke-exposure as used to assess the effect of extended exposure to whole and filtered cigarette smoke on bacterial clearance. In this manner, antibacterial activity will be assessed immediately following and 24 hours after exposure to cigarette smoke for 1, 5 and 15 days. Under these conditions it will be possible to compare the effect of whole and gas-phase cigarette smoke on the rate of ingestion and destruction of inhaled bacteria by alveolar macrophages and to assess the relative influence of the particulate and gas phases of smoke on these important parameters of macrophage function.

(2) In Vitro Phagocytosis and Intracellular Killing Activity: In a separate series of studies, to determine the percent bacteria phagocytized, percent intracellular survival of phagocytized bacteria and percent bacteria killed by macrophages, alveolar macrophages harvested from smoke-exposed and control mice and rabbits will be challenged with S. aureus in an in vitro phagocytosis system. Fixed numbers of staphylococci will be added to known numbers of macrophages adhering to the flat surface of a tissue culture flask containing Hanks' solution (0.1% glucose). Immediately after bacterial challenge and at various intervals thereafter, the extracellular fraction containing free or unphagocytized bacteria will be isolated by removing the supernatant fluid from macrophage cultures with a pipette. To recover the macrophage fraction, the tissue culture flask will be vigorously shaken after adding glass beads and distilled water. Bacterial counts will be obtained from each fraction by a standard pour plate technique. The in vitro phagocytosis system and method of processing macrophage cultures used to obtain this information is presented in detail on page 23 (Addendum I).

Since phagocytosis has been shown to be a two stage event consisting of the adherence to and subsequent destruction of ingested bacteria by phagocytes, methods will also be used to differentiate bacterial attachment from ingestion. After challenging macrophages cultures with S. aureus for 1.5 hrs, the macrophage fraction will be separated from the extracellular fraction as previously described and incubated for 15 min in 3.0 ml of Hanks' solution containing 2.5% trypsin (Grand Island Biological Company). The bacteria released from the surface of macrophages by trypsin (adherence fraction) will be recovered by decanting the supernatant and washing the macrophages three times with 2.5 ml of Hanks' solution. The bacteria associated with the trypsin-treated macrophages are present within macrophages and may be referred to as the intracellular fraction of the total bacteria recovered. To recover the intracellular fraction of bacteria, the trypsin-treated macrophages will be lysed by treatment with cold sterile distilled water. The number of viable bacteria present in the adherence and intracellular fractions is determined from bacterial counts obtained from nutrient agar pour plates incubated at 37°C for 48 hrs. Under these conditions, it is possible to determine the % viable bacteria attached to (% adherence) and within alveolar macrophages (% intracellular) at various times during the phagocytic event:

$$\% \text{ adherence} = \frac{\text{No. viable bacteria in adherence fraction}}{\text{No. viable bacteria in the adherence and intracellular fractions}} \times 100$$

$$\% \text{ intracellular} = \frac{\text{No. viable bacteria in the intracellular fraction}}{\text{No. viable bacteria in the adherence and intracellular fractions}} \times 100$$

In other studies, intracellular destruction of S. aureus by alveolar macrophages will be studied in the absence of all other aspects of the phagocytic event. For this purpose lysostaphin (Schwartz Mann) will be used (final concentration of 150 µg/ml) to kill all extracellular bacteria as well as those adhering to macrophage membranes. Lysostaphin is a muralytic enzyme which does not enter phagocytes and selectively eliminates extracellular staphylococci (Tan et al, J. Lab.Clin.Med., 78:316, 1971). Macrophage cultures are challenged with S. aureus for 1 hr at 37°C. At this time, 0.1 ml of lysostaphin is added to all macrophage cultures. After 30 minutes incubation at 37°C, duplicate flasks will be removed from the incubator and treated with 0.3 ml of trypsin to inactivate and neutralize the lysostaphin. Viable intracellular bacteria will be released by agitation with glass beads and osmolysis with cold sterile distilled water. Two hours later, duplicate flasks will be taken out of the incubator as outlined above. Viable bacterial counts will be made 48 hrs later and the % intracellular killing of S. aureus is determined as follows:

$$\% \text{ intracellular killing} = 100\% - \left[\frac{\text{No. viable bacteria in macrophage cultures 2.0 hrs after bacterial challenge period}}{\text{No. viable bacteria in macrophage cultures immediately after bacterial challenge period}} \times 100 \right]$$

(3) Influence of Other Factors: Studies are also planned to evaluate the influence of increased macrophage numbers, opsonization of bacteria, pre-treatment of bacteria with alveolar lining material, pre-treatment of bacteria with the total acellular fraction of lung harvests on the phagocytic and bactericidal powers of alveolar macrophages. In separate studies alveolar macrophages will be challenged with S. aureus and incubated at 37°C in tissue culture flasks containing Hanks' solution supplemented with the following: (a) normal serum with and without specific immune serum added, (b) alveolar lining material (ALM) alone, ALM plus normal and immune serum, ALM plus immune serum and ALM plus normal serum and (c) concentrated acellular fraction (CAF) alone, CAF plus normal and immune serum, CAF plus immune serum and CAF plus normal serum. This information is needed for proper analysis of the events observed, under in vivo conditions following an airborne bacterial challenge and is in keeping with recent observations suggesting that local immune systems and pulmonary secretions play a significant role in pulmonary defense (1,2) and, as such, may act as mediators of alveolar macrophage function. In addition, the above information will be used to establish in vitro culture conditions necessary for meaningful studies of macrophage mobilization and the metabolic activity of alveolar macrophages as outlined below; namely, in vitro culture conditions that best reflect the in vivo circumstances in the live and intact lung. (See Methods E., pgs. 2A9-10).

(4) In Vitro Studies of Cell Adhesiveness and the Motility and Migratory Response of Basal and Mobilized Alveolar Macrophages to Chemotactic Substances: An understanding of this aspect of alveolar macrophage activity is desirable because motility and changes in cell adherence may be critical in the mobilization and migration of lung phagocytes to pulmonary sites, as well as their subsequent antibacterial activity during the normal situation and in response to the inhalation of viable bacteria and cigarette smoke. The data obtained from these studies will be correlated with the results of studies completed in this laboratory concerning the mobilization of alveolar macrophages in response to the inhalation of viable bacteria in the normal situation and during the inhalation of cigarette smoke.

For this purpose, a method of studying leukocyte motility

reported by Carruthers (3) will be adapted to alveolar macrophages. This method is based on the ability of motile cells to move through the pores of a membrane filter. Two O-ring joints separated by a millipore filter are clamped together and sealed to form 2 distinct chambers. Fixed numbers of alveolar macrophages ($1\text{-}3 \times 10^7$) suspended in Hanks' solution (3 ml.) containing glucose will be introduced into chamber number one which is then sealed with a paraffin plug. The second chamber will be filled with 5 mg. of insoluble potato starch and plugged. After an initial period of incubation at 37°C to permit monolayer formation, the chambers will be inverted so that the test cells will then be on the bottom side of the filter, and the chemotactic substance, if present, is on the top side of the filter. The chamber will then be placed in an incubator at 37°C for 4 hours. At the end of various hourly intervals, the filter will be removed, stained with hematoxylin and subjected to microscopic study. In this way, the number of cells on both sides of the filter will be enumerated and used as an index of the migratory response elicited by stimulatory agents. Separate studies are planned to evaluate the changes in cell adhesiveness that occur in phagocytizing alveolar macrophages. The method of studying the adhesive properties of blood leukocytes reported by Allison and Lancaster (4) will be adapted to alveolar macrophages. Test tube cultures of fixed numbers of lung phagocytes suspended in Hanks' solution will be challenged with known numbers of bacteria. Under conditions favoring maximum phagocytosis, microscopic methods will be used to determine the formation of cell aggregates by phagocytizing macrophages. Changes in cell adhesiveness will be evaluated under the same experimental conditions described above to assess motility.

(5) Metabolic Studies: The proposed project will include studies of the energy metabolism, hydrolytic enzyme activity and peroxidative metabolism of basal and mobilized alveolar macrophages harvested from control and smoke-exposed animals. This information is desirable, since energy output and cell metabolism represent important potential links in the successful mobilization and subsequent antibacterial activity of alveolar macrophages. Indeed, alterations in hydrolytic enzyme activity and peroxidative metabolism may interfere with the primary immunological function of macrophages in pulmonary defense, namely, the destruction of inhaled microorganisms. The metabolic studies will be performed under the same culture conditions (suspending medium) used to assess the influence of cigarette smoke on the phagocytic and bactericidal capacities of alveolar macrophages.

Respiration studies: Experiments are planned which will permit determinations of the oxygen consumption and lactic acid content of alveolar macrophages incubated in basal medium and media supplemented with glucose plus serum with and without the presence of particles that induce phagocytosis. In separate studies serum will be supplemented with and/or replaced by alveolar lining material and the concentrated acellular fraction of lung harvests as outlined in the phagocytosis studies detailed on pgs.2A9-10. These studies will be performed with the following categories of macrophages: (a) basal macrophages harvested from control and smoke-exposed animals and (b) mobilized macrophages harvested from control and smoke-exposed animals immediately after exposure to aerosols of a phosphate buffer for 30 minutes. (see Methods of Procedure p.2A-10).

Glucose metabolism studies: Experiments with specifically labelled glucose as substrate will be performed to evaluate the effect of cigarette smoke on the metabolism of glucose by alveolar macrophages. These studies will be done with the same categories of basal and mobilized macrophages used in the respiration studies (see Methods of Procedure p. 2A-10).

ATPase activity: Experiments are planned to compare the ATPase activity of basal and mobilized alveolar macrophages harvested from control and smoke-exposed animals. ATPase activity will be determined from the liberation of inorganic phosphate (Pi) upon incubation of alveolar macrophages with adenosine triphosphate (ATP). (See Methods of Procedure p. 2A-10)

Catalase activity, peroxidase activity and hydrogen peroxide production: Recent studies (5) have presented evidence for the presence of a catalase-dependent peroxidative metabolism. Peroxidative metabolism represents a biochemical pathway capable of increasing glucose metabolism and hydrogen peroxide formation. In this regard, published reports clearly demonstrate that phagocytosis by normal alveolar macrophages is accompanied by increased glucose metabolism (6,7) and intracellular recovery of hydrogen peroxide (5). The potential role of catalase controlled concentrations of hydrogen peroxide as an intracellular bactericidal agent has been recognized. For these reasons studies are proposed to compare the catalase activity, peroxidase activity and hydrogen peroxide production in macrophages harvested from control and smoke-exposed rabbits. These studies will be performed with basal macrophages, and macrophages mobilized in response to the inhalation of heat-killed staphylococci harvested from both control and smoke-exposed animals. (See Methods of Procedure p. 2A-11)

Hydrolytic enzyme studies: The proposed project will include a study of the enzymatic activity and intracellular distribution of a specific group of hydrolytic enzymes in basal and mobilized macrophages harvested from control and smoke-exposed animals. The enzymes acid phosphatase, lysozyme, lipase, beta-glucuronidase and cathepsin are of immediate interest because their activity is increased in the BCG induced alveolar macrophage (8,9). To accomplish these aims, the enzymatic activity detectable in the cell-free supernatant and alveolar macrophage fractions of lung harvests will be assayed. Interest in both fractions of lung harvests resides in the fact that bronchial mucus contains several poorly defined substances (10) including lysozyme (11) that exert nonspecific bacteriostatic and bactericidal activity against gram positive and gram negative bacteria as well as the potential phagocytosis promoting factor present in pulmonary secretions (1,2). In addition, alveolar macrophages by virtue of their high hydrolytic enzyme content, presence in large numbers and rapid turnover rate may contribute to the enzymatic activity found in mucus secretions.

A comparison will be made of the enzymatic activity and intracellular distribution of the above hydrolases in the following categories of macrophages: (a) basal macrophages harvested from control and smoke-exposed animals and (b) mobilized macrophages harvested from control and smoke-exposed animals immediately after exposure to aerosols of viable or heat-killed staphylococci. For this purpose, smoke-exposed and control animals will be exposed to the bacterial aerosols for 30 minutes. Enzyme determinations will be made with the lung harvests obtained from both groups of animals immediately after bacterial challenge and at hourly intervals over a 4 hour post-aerosol exposure period. The choice of laboratory animals (mice or rabbits) to be used in all enzyme studies will be governed by the minimal yields necessary for adequate enzyme assay. (See Methods of Procedure pgs. 2A-11-12)

Experimental Methods:

A. Smoke-Exposure System: A smoke generating apparatus will be used to deliver puffed cigarette smoke under controlled conditions. In the studies proposed herein, the apparatus will be adjusted to deliver 35 ml puffs each of 2 seconds duration from non-filtered cigarettes. The instrument is equipped with a rotating disc which can accommodate 30 cigarettes at one time, so that it is possible to maintain a continuous stream of puffed smoke generated at a rate of 1 L/min. The initial smoke delivered by the cigarettes is diluted approximately 1 to 20 and transported across an animal exposure chamber by a secondary air flow. Control animals are placed in a plexiglass chamber and exposed to a secondary air flow. These are the same conditions of smoke exposure that were used to assess the influence of acute and extended exposure to puffed cigarette smoke on bacterial clearance and alveolar macrophage function in studies reported under Addendum I, Supporting Data, pages 21 to 30. In studies with filtered cigarette smoke, the smoke generating apparatus will be modified to include the insertion of a glass fiber filter disc. The tar content in the smoke will be determined by established methods. Air samples from the smoke exposure chamber will be collected on filter paper, weighed, extracted in alcohol and subjected to spectrophotometric assay.

B. Conditions of Animal Smoke-Exposure: Animals will be exposed daily for 1.0 hr for 3 weeks to whole cigarette smoke or cigarette smoke passed through a glass fiber disc. The maximum number of total days of smoke-exposure planned over the entire 3 week study period will be 15 days. However, total days or daily length of smoke-exposure will be reduced if toxicity, animal death or bacterial contamination of broncho-pulmonary tissue are noted. The daily length of exposure corresponds to the smoke-exposure periods previously used in this laboratory to study the effect of acute and extended exposure to cigarette smoke on tracheal clearance, lung clearance and alveolar macrophage activity. Under these conditions, it will be possible to determine the effect of whole and gas-vapor phase cigarette smoke on pulmonary defense and to correlate these observations with the data obtained to date under conditions of acute and extended exposure to cigarette smoke (See Addendum I, Supporting Data, pages 21 and 22.). The proposed protocol is similar to that used by LaBelle et al (12) to study the effects of acute and extended exposure to cigarette smoke on pulmonary clearance of radioactive test particles.

C. Bacterial Aerosol Exposure Unit: Since bacterial clearance studies comprise a major part of the proposed research project, a review of the method of aerosol formation, exposure and quantitative aspects of bacterial clearance is indicated. Aerosols are generated from a buffered suspension (pH 7.3) of staphylococci contained in glass nebulizers. The initial spray from the nebulizers is directed into mixing chambers through which a secondary air flow of 100 cubic feet/min is drawn. The large volume of secondary air serves to mix, dilute and dry the initial bacterial aerosol; direct it past an interposed baffle for removal of large droplets; and then carry it through a large plexiglass exposure chamber that can accommodate up to 200 mice. The particle size distribution of the bacterial aerosol is monitored with an Andersen Sampler (13). White male Swiss Webster mice are divided into groups of 5 to 10 animals and exposed to staphylococcal aerosols for 30 min. Immediately after exposure (0 time) one group of animals is sacrificed to determine the numbers of viable bacteria deposited in the trachea and lungs, respectively. The trachea and lungs are removed as separate blocks and individually ground in glass homogenizers. The remaining groups of challenged mice are killed and processed 15 min, 30 min, 1 hr, 2 hrs and 4 hrs after aerosol exposure. Bacterial counts are obtained from nutrient agar pour plates of lung and tracheal tissue homogenates. By this method paired studies of lung and tracheal clearance of bacterial deposits are possible. By subtracting the mean number of culturable bacteria retained in

the lung at each interval during the post aerosol exposure period from the number originally deposited (0 time), the mean number of staphylococci killed by the lung may be derived. From these data lung clearance rates are calculated by expressing the number retained in the lung as a percentage of the deposition number (0 time) and subtracting this value from 100%. Similarly, from the numbers of viable bacteria present in the trachea at 0 time and at the same intervals after aerosol exposure, the numbers and percent bacteria cleared by the trachea may be obtained. It must be emphasized that the term clearance refers solely to the decline in the number of culturable bacteria remaining in the trachea and lungs after aerosol exposure.

D. Harvesting of Alveolar Macrophages: In order to correlate macrophage activity with bacterial clearance, a method has been developed in this laboratory for harvesting alveolar macrophages from the murine lung (14,15). Mice are sacrificed and the trachea and lungs exposed. The intact lung is washed by 5 successive 1.0 ml washes with Hanks' solution and the harvested cells recovered by centrifugation. By this method, it is possible to obtain 1.0 to 2.0 x 10^5 macrophages from each mouse lung with 90% viability. Total cell counts are performed in a bright line hemocytometer and differential counts are made on Wright stained smears. Cell viability is assessed by the capacity of alveolar macrophages to reject the stain Eosin Y. The number of alveolar macrophages that are available in harvest by this technique under basal conditions is referred to as the basal yield. Therefore, mobilization is taken to represent the increase in macrophage numbers over basal levels harvestable from the lungs after a bacterial challenge. The mobilization of alveolar macrophages may be quantitated by this technique. Studies completed in our laboratory to date indicate that macrophage yields are increased 1.5 times basal levels after exposure to aerosols of a phosphate buffer or dead Staphylococcus aureus (14), and 2 to 3 times basal levels in response to aerosols of viable S. aureus (14,15). During the post-aerosol exposure period there is an initial drop in macrophage numbers but elevated levels are restored in 30 min. and this increase is maintained for 4 hours (15). (See Addendum II, publication in press entitled "Clearance of Inhaled Bacteria from the Murine Respiratory Tract")

Alveolar macrophages are harvested from the lungs of albino rabbits weighing 1.0 to 2.0 kilograms by the general method of Myrvik et al (16). The animals are killed by injecting air in the marginal ear vein. This method of sacrificing animals is used to avoid any depressant effects that anesthetics may have on alveolar macrophage function. The trachea and lungs are exposed, and macrophages harvested by washing out the intact lungs with 17 ml. of Hanks' solution. The harvested cellular contents of the lungs are recovered by centrifugation at 2000 rpm for 20 minutes. Total cell counts are performed in a bright line hemocytometer, and differential counts are made on Wright stain smears. By this method, 95% of the macrophages harvested are viable as determined by the Eosin Y technique(17).

E. Acellular Fractions of Lung Harvests: Recent studies suggest that secreted fluids present in the bronchopulmonary tree may independently or in concert with alveolar macrophages play a significant role in pulmonary defense against inhaled bacteria. Alveolar lining material (ALM) has been reported to enhance the bactericidal activity of rat alveolar macrophages (1). Secretory IgA is present in the tracheobronchial washing of normal patients (2) and has the capacity to kill Escherichia coli (18) and inhibit the adherence of certain strains of streptococci to epithelial tissue (19). For these reasons, the acellular fractions of lung harvests (ACF) will be collected, concentrated, Secretory IgA levels monitored and ALM isolated. In addition, the antibacterial and phagocytic properties of concentrated acellular fraction and ALM against S. aureus will be assessed in the phagocytosis system detailed on page 23. These studies will be performed with acellular fractions of lung harvests obtained from control and smoke-exposed animals.

The method of LaForce et al (1) will be used to isolate alveolar lining material from the lungs of rabbits. Rabbits are sacrificed by injecting air into the marginal ear vein, and the trachea and lungs are exposed. The trachea is canulated with a sterile polyethylene tube, and 17 ml of sterile heparinized saline (10 units per ml) are introduced in the lung and recovered by aspiration. The recovered bronchoalveolar saline lavage fluid is centrifuged at 800 g for 8 minutes; the supernatant decanted and saved. The cell free supernatant is centrifuged at 40,000 g at 4°C for 20 minutes. The recovered precipitated pellet represents the alveolar lining material or surfactant fraction (20).

In order to study the antibacterial and phagocytosis promoting characteristics of the entire acellular fraction of lung harvests, bronchoalveolar saline lavage fluid recovered from rabbit lungs will be initially concentrated by ultrafiltration (Diaflow Membranes, Amicon Corp) and fractionated by gel column chromatography.

The presence of Secretory IgA (SIgA) in bronchoalveolar washings will be monitored by double immunodiffusion (21) against anti-SIgA and anti-secretory piece sera. SIgA levels will be quantitated by single racial immunodiffusion (22) utilizing anti-SIgA serum impregnated in the agar gel and SIgA as the antigen standard. The SIgA antigen standard will be prepared from clarified colostrum (23). The latter procedure includes: (a) separation by gel chromatography and further purification by anion-exchange chromatography using a stepwise elution gradient of phosphate buffers of varying ionic strengths. The purity of SIgA will be assessed by disc electrophoresis (24).

F. Metabolic Studies: Areas of comparative assays to be performed.

Respiration: Measurements of oxygen uptake will be determined in a Gilson respirometer using flasks with a 15 ml capacity containing monolayers of alveolar macrophages in a total liquid volume of 3.2 ml (25). The CO_2 will be absorbed by 0.2 ml of 20% KOH in the center well. In accordance with the protocol of each study, glucose (5.0 to 10 mM) and polystrene spheres at a concentration of 2.0 to 2.5 mg/ml will be introduced via the side arm. After completion of the oxygen consumption measurements, the cells will be harvested and their lactic acid content determined by the method of Barker et al(26). For this purpose, the recovered macrophages will be washed in saline and cell extracts are to be prepared as described by Myrvik et al (27).

Glucose metabolism: The radioactive measurements will be done as described by Myrvik et al (27). Alveolar macrophages are harvested and placed in Erlenmeyer flasks containing Medium 199 without serum or glucose. In separate experiments BCG and heat killed staphylococci will be added to all flasks except the control flasks. After incubation at 37°C in a shaker bath for 1, 2, 4, 6 and 18 hours, glucose 1-C^{14} or glucose 6-C^{14} is added to the flasks, and reincubated for 1 hour. The reaction is stopped with sulfuric acid and counts obtained with a liquid scintilation counter.

ATPase: ATPase activities will be carried out by the method outlined by Wahler et al (28). The assay medium will contain 100 mM sucrose, 30 mM glyclglycine, 30 mM imidazole, 5 mM $MgCl_2$, 2 mM ATP, protein equivalent of cells 50 to 100 ug and, as indicated, 50 mM NaCl plus 5 mM KCL (pH 7.5). In studies performed in the absence of Na^+ and K^+, the medium will contain 200 mM sucrose. Reactions will be carried out in a 2nd volume for 20 minutes at 30°C and activity will be expressed as micromoles of Pi liberated per milligram of protein per hour.

Catalase/Peroxidase Activites and H_2O_2 production: Catalase activity will be determined by the method of Feinstein (29) using 0.1M sodium perborate as substrate. Perborate utilization in 5 min is measured by titration with a 0.1N solution of potassium permanganate after the reaction is stopped with 1N sulfuric acid. Catalase activity will be determined after incubation in Krebs Ringer Phosphate Solution containing 15% homologous serum and 5.5mM glucose at pH 7.4. Measurements will be performed on macrophage preparation after disruption by either homogenization or repeated freeze-thawing using acetone-dry ice. Both total extract and supernatant obtained by centrifugation at 8000 rpm for 10 min will be assayed. Activity will be expressed as milliequivalents of perborate utilized in 5 min, 1 U representing the utilization of 1 mEq of perborate. Peroxidase activity will be assessed by a modification of the guaiacol method of Chance and Maehly (30). Whole extracts of freeze-thawed cells will be employed. The assay medium contains 0.1 M phosphate buffer at pH 7.4, 0.5 ml of 100 mM guaiacol, 0.2 ml of extract sample, and 0.02 ml of ice-cold 10 mM H_2O_2. Absorbancy changes due to tetraguaiacol formation will be measured at 750 nm in a spectrophotometer and the time required to produce an 0.05 U increase in absorbancy recorded. Results will be expressed in reciprocal seconds per 10^9 cells.

Hydrogen peroxide will be determined spectrophotometrically on dialysates of AM as described by Paul and Shara (31). The nonfluorescent dye, diacetyl-2,7-dichlorofluorescin (LDADCF), was synthesized by the method of Brandt and Keston (32) and the fluorescence of the oxidized product of alkali-activated LDADCF was measured with an Aminco-Bowman spectrofluorimeter. The excitation wave length was 340 nm and the emission wave length 525 nm.

Hydrolytic Enzymes: The lung washings obtained from animals by the lavage technique will be separated into a cell-free supernatant fraction and cellular fraction containing alveolar macrophages by centrifugation at 4°C for 15 min at 10,000 rpm (11). The supernatant will be frozen at -60°C and stored until assay. The harvested alveolar macrophages will be washed twice in phosphate buffered saline (pH 7.2); quantitated by hemocytometer count; and viability assessed. Saline extracts of the washed cells are to be prepared for enzyme activity studies as outlined by Myrvik et al (27) cell disruption by alternate freezing and thawing for 5 consecutive cycles and removal of cellular debris by centrifugation at 2,500 rpm for 10 min at 4°C. The completeness of cell disruption will be audited by phase optics. In order to evaluate the contribution made by alveolar macrophages to the enzymatic activity detectable in the supernatant fraction, in vitro studies of the rate of release of the enzymes in question by pulmonary macrophages are planned. As outlined by Holzman et al (11) large numbers of alveolar macrophages will be suspended in tissue culture medium and samples of the cell population assayed for specific enzymatic activity after incubation at 37°C for various periods of time. In studies of the intracellular distribution of the hydrolases, alveolar macrophages will be suspended in a 0.25M sucrose solution, ruptured by homogenization and sedimented by centrifugation into four fractions (33). The nuclear fraction (N) will be sedimented by centrifugation at 250 X g; the heavy granule fraction (HG) at 5000 X g for 15 min; and the light granular fraction (LG) and supernatant fraction (S) by centrifugation at 25,000 X G. Each fraction will be submitted to 5 cycles of freezing and thawing and clarified by centrifugation at 2500 X G for 20 min and subjected to enzyme assay.

For the purpose of enzyme analysis, lysozymes will be quantitated by using suspensions of Micrococcus leisodykticus as substrate. Tests will be standardized with known amounts of crystalline egg white lysozyme and results expressed as egg white lysozyme equivalents. Acid phosphatase will be measured by the procedure of Hofstec(34) using O-carboxyphenyl phosphate as substrate. An increase in absorbance of 0.001 optical density units/min under standard conditions

will be taken to represent a unit of activity. Beta-glucuronidase will be assayed by the procedure of Fishman et al (35) using phenolphthalein mono-betaglucuronide as substrate with reactions carried out in 0.1M acetate buffer at pH 4.5 and 38°C. One unit of glucuronidase is the activity resulting in the liberation of 1 ug/hr phenolphtalein. Lipase activity will be determined by the method of Cohn et al (8) in which naphthol laurate serves as substrate and the increase in activity caused by sodium taurocholate is taken to represent minimal lipase activity. The results are to be expressed as micromoles of naphthol liberated per hour. Cathepsin will be assayed employing a 2% solution of denatured hemoglobin as a substrate, as described by Anson (36) and protein digestion estimated with a spectrophotometer by absorption at 280 nm (33). A unit of activity being defined as the increase in optical density produced by 0.001 meq of tyrosine.

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2. Kelmowitz, R. : Immunoglobulins in normal tracheo-bronchial washings. J. Lab. Clin. Med. 63:54, 1964.

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9. Cohn, Z. A. and Wiener, E.: The particulate hydrolases of macrophages. II. Biochemical and morphological responses to particle ingestion. J. Exp. Med. 118: 1009, 1963.

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12. LaBelle, C.W., Bevilaqua, D.M. and Breiger, H.: The influence of cigarette smoke on lung clearance: Arch. Environ. Health 40: 588, 1966.

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14. Guarneri, J. J. and Laurenzi, G.A.: Mobilization of alveolar macrophages as a pulmonary defense mechanism against inhaled bacteria.: Bacteriol Proc., p. 100, 1968.

15. Guarneri, J. J. and Laurenzi, G.A.: Effect of alcohol on the mobilization of alveolar macrophages: J. Lab. Clin. Med. 72: 40, 1968.

16. Myrvik, Q. N., Leake, E.S., Fariss, B.: Studies on pulmonary alveolar macrophages from the normal rabbit: A technique to procure them in a high state of purity. J. Immunol. 86: 128, 1961.

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17. Dannenberg, A. M., Burstone, M. S., Walter, P. C., and Kinsley, J.W.: A histochemical study of phagocytic and enzymatic functions of rabbit mononuclear and polymorphonuclear exudate cells and alveolar macrophages. I. Survey and quantitation of enzymes, and states of cellular activation.: J. Cell. Biol. 17:465, 1963.

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20. Morgan, T.: Pulmonary Surfactant. N. Eng. J. Med. 284:1185, 1971.

21. Oucherlony, O.: Handbook of Immunodiffusion and Immochlectrophoresis. Ann Arbor Science Publications. Ann Arbor, Michigan, 1968.

22. Fahey, J. L. and E. M. McKelvey: Quantitation of serum immunoglobulins in antibody - agar plates. J. Immunol. 8:335, 1965.

23. Cebra, J. and Robbins, J.: Gamma A immunoglobulin from rabbit colostrum. J. Immunol. 92: 12, 1966.

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26. Barker, S.B. and Summerson, W. H.: The colormetric determination of lacetic acid in biological materials. J. Biol. Chem. 138: 535, 1941.

27. Myrvik, Q. N. and Evans, D.G.: Metabolic and immunological activities of alveolar macrophages. Environ. Health. 14: 92, 1967.

28. Wahler, B. E. and Wollenberg, A.: Zur Bestimmungdes orthophospats neben sancremolybdat - labelen phosphoroaureverbindungen. Biochem Z.329: 508, 1958.

29. Feinstein, R. N.: Perborate as substrate in a new assay of catalase. J. Biol. Chem. 180: 1197, 1949.

30. Chance, B. and Maehly, A.C.: Assay of catalases and peroxidase. In Methods in Enzymology. S. Colowick, editor. Academic Press, Inc. N.Y. 2:764, 1955.

31. Paul, B. and Sbarra, A.J.: The role of the phagocyte in host-parasite interactions. XIII. The direct quantitative estimations of H_2O_2 in phagocytizing cells. Biochem. Biophys. Acta. 156: 168, 1968.

32. Brandt, R. and Keston, A.S.: Synthesis of diacetyldichloro-fluorescin: A stable reagent for fluoremetric analysis. Anal. Biochem. 11:6, 1965.

33. Heise, E.R., Myrvik, Q.N. and Lealse, E.S.: Effects of Bacillus Calmette Guerin on the levels of acid phosphatase, lysozyme and cathepsin in rabbit alveolar macrophages. J. Immunol. 95: 125, 1965.

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34. Hofstee, B.H.J.: Direct and continuous spectrophotometric assay of phosphomonesterases. Arch. Bioch. 51: 139, 1954.

35. Fishman, W.H., Springer, B. and Bruenetti, R.: Application of improved glucuronidase assay method to study of human blood B - glucuronidase. J. Biol. Chem. 173: 449, 1948.

36. Anson, M. L.: The estimations of pepsin, trypsin, papain and cathepsin with hemoglobin, J. Gen. Physiol. 22: 79, 1938.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The Microbiology Research Laboratories are located as a complex in the Triboro Hospital at Queens Hospital Center, Jamaica, New York. They include: (a) an aerosol exposure laboratory, (b) a smoke-exposure laboratory, (c) individual laboratories for macrophage studies and microbiology, (d) 2 rooms for storage space and refrigerators. Animal quarters are provided in another area of the hospital. The entire laboratory area occupies approximately 700 square feet.

The equipment in this area includes a complete bacterial aerosol generating and exposure system with mixing chambers and decontaminating units, and an Anderson apparatus for measuring particle size of bacterial aerosols: A cigarette smoke generating apparatus and exposure chamber and a sequential sampler and gas liquid chromatography unit for determining concentration of the particulate and gas phase of cigarette smoke. Other major instrumentation present include the following: (a) standard microscopes, (b) 1 infusion pump, (c) a centrifuge, (d) 2 large refrigerators, (e) sonic dismembranator, (f) 2 water baths and shaker, (g) 1 freezer, (h) 2 incubators and 1 environmental chamber, (i) 4 vacuum pumps, (j) pH meter, (k) Beckman DU2 recording spectrophotometer, (l) spectronic 20 spectrophotometer, (m) Gilson respirometer, (n) a lyophilizer unit, (o) immuno and disc electrophoresis apparatus, (p) large autoclave, (q) analytical balance and (r) flash evaporator. There is also equipment for qualitative and quantitative bacteriology studies, tissue homogenation, administration of gas mixtures and animal surgery. A Revco deep freezer (-75°C) is also available. High performance scintillation counters capable of isotope work are present in the hospital and available for research use. In addition, a fully equipped laboratory capable of performing histological and electron microscopy studies are available to the Division of Microbiology.

11. Additional facilities required:

None.

12. Biographical sketches of investigator(s) and other professional personnel (append):

See Pages 6-13

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See Pages 14-15

4.

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Joseph J. Guarneri, Ph.D. Principal Investigator	20 hrs/60 hrs	
Boris A. Shidlovsky, Ph.D.	7 hrs	
Research Associate, Ph.D. (to be recruited)	100% Fringe Benefits	
Technical		
Research Technician (to be recruited)	100%	
	Sub-Total for A	

REDACTED

B. Consumable supplies (by major categories)

See page 4A for detailed list

Sub-Total for B	5,150

C. Other expenses (itemize)

Travel	900
Publications	100

Sub-Total for C	1,000
Running Total of A + B + C	35,485

D. Permanent equipment (itemize)

Sorvall RC 5 Automatic Superspeed
Refrigerated Centrifuge including heads ($4905)

Sub-Total for D	4,905
E	5,323
Total request	$45,712

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	5,200	1,000	1,000	5,683	$41,565
Year 3		5,200	1,000	500	6,063	$46,980

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B. Consumable Supplies

1.	Cigarettes for smoke studies	$1,000
2.	Mice Caesarian delivered	1,000
3.	Rabbits	1,200
4.	Immunodiffusion Plates, Anti SIgA and Anti Secretory Piece Sera, Radial Immunodiffusion kit and templates	200
5.	Chromatography columns with 4 way valve, accessories, and Reagents for Chromatography, Sephadex G 200, Acrylamide kit, DEAE	303
6.	Reagents and curvettes for enzyme studies and flasks and accessories for respiration studies	500
7.	Tissue culture glassware, tissue culture media and bacteriology media, and nebulizers.	547
8.	Isotopes uniformly labelled glucose - 1-C^{14} and glucose 6-C^{14}	300
9.	Petri dishes and plastic disposables	100
	Total	$5,150

D. Permanent Equipment (Justification)

1. The Sorval RC 5 Superspeed Refrigerated Centrifuge is needed for the alveolar lining material studies (ALM) proposed on pages 2A-9 and 2A-10 of grant request. The instrument and its rotors permit centrifugation up to 49,500 g with controlled temperature. The latter permits g forces and controlled conditions necessary for the isolation of ALM without the loss of biological activity.

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophages and Mucociliary Function.	The Council for Tobacco Research, U.S.A. Grant Nos. 547C, 547 CR-1 and 547 CR-2	$65,152	7/1/71 to 6/30/75
Important Determinants of Pulmonary Resistance to Infection, Alcoholic Intoxications.	Long Island Jewish-Hillside Medical Center Grant No. 274	22,413	5/3/74 to 6/30/75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Joseph J. Guarneri, Ph.D.

Signature [redacted] Date 1/21/75

Telephone _____
Area Code Number Extension

Checks payable to

ng Island Jewish-Hillside Medical Center

Mailing address for checks

Mrs. Eva Meyer, Grant Manager
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11040

Responsible officer of institution

Typed Name Harold L. Light

Title Deputy Director

Signature _____ Date 1/21/75

Telephone 212-343-6700 (Ext.2723)
Area Code Number Extension

10035463332

12 Biographical Sketch Joseph J. Guarneri, Ph.D. Principal Investigator

NAME: Joseph J. Guarneri, Ph.D.

ADDRESS: HOME: REDACTED

OFFICE: Long Island Jewish-Hillside Medical Center/Queens Hospital Center
82-68 164th St., Jamaica, New York 11432

TELEPHONE: REDACTED
HOME:
OFFICE: 212-990-2335

BIRTHDATE AND PLACE: REDACTED

CITIZENSHIP: REDACTED

MARITAL STATUS: REDACTED

SOCIAL SECURITY #: REDACTED

EDUCATION: REDACTED
- New York University; B.A. (Biology), 6/49
- Saint John's University; M.S. (Microbiology), 6/63
REDACTED
- Saint John's University; Ph.D. (Microbiology), 6/66

INTERNSHIP RESIDENCY: Not applicable.

MILITARY SERVICE: 1/49 - 3/46 - Sergeant, U.S. Army; Medical Corp., Camp Lee, Virginia

BOARD STATUS: American Society for Microbiology; Certification as Specialist in Public Health and Medical Laboratory Microbiology.
American Academy of Microbiology; Fellowship (Pending).

TYPE OF PRACTICE: Not applicable.

LICENSURE STATUS: Certificate of Qualification for Director of a Clinical Microbiology Laboratory, City of New York, Department of Health.

1003546333

ACADEMIC POSITIONS:

9/61 - 6/66 - Research Associate, Division of Respiratory Diseases, New Jersey College of Medicine and Dentistry.

6/66 - 6/68 - Instructor in Medicine, Department of Medicine, New Jersey College of Medicine and Dentistry, Jersey City, New Jersey.

7/68 - 1/72 Director, Pulmonary Aerobiology Research Laboratory, Division of Infectious Diseases, Department of Medicine, Saint Vincent Hospital, Worcester, Mass.

ACADEMIC POSITIONS:

1/72	Attending Microbiologist, Long Island Jewish-Hillside Medical Center/Queens Hospital Center.
6/73	Associate Clinical Professor Pathology, SUNY at Stony Brook.
6/73	Coordinator Allied Health Sciences, Queens Hospital Center, Jamaica, New York.
9/73	Associate in Microbiology, St. John's University, Jamaica, New York.

MEMBERSHIP IN PROFESSIONAL SOCIETIES:

REDACTED

REDACTED

REDACTED

REDACTED

HONORS AND AWARDS:

Sigma XI, Saint John's University, 1963

3/68 to 5/69 - Public Health Service. H.I.H. Award #A.I. 08963-01.
Title: The Mechanism of Pulmonary Resistance to Infection. Principal Investigators: G.A. Laurenzi, M.D. Associate Director: J.J. Guarneri, Ph.D. - Amount: $ 67,769.

7/68 to 6/69 - The Council for Tobacco Research-U.S.A. Grant Award #547. Title: The Effect of Cigarette Smoke on the Nature and Function of Alveolar Macrophages. Principal Investigator: G.A. Laurenzi, M.D. Co-Investigator: J. J. Guarneri, Ph.D. - Amount $36,135.

9/68 to 6/71 - Saint Vincent Hospital Research Foundation. Title: The Role of the Alveolar Macrophage in Pulmonary Defense Against Inhaled Bacteria. Principal Investigator: J. J. Guarneri, Ph.D. Amount: $ 18,667.

6/69 to 5/72 - Public Health Service. H.I.A. Award #AI 08963-02. Title: The Mechanism of Pulmonary Defense Against Infection. Principal Investigator: G.A. Laurenzi, M.D. Associate Director: J.J. Guarneri, Ph.D. - Amount: $128,137.

1003546334

HONORS AND AWARDS:

7/69 - 6/71 - The Council for Tobacco Research - U.S.A. Grant Award #547BR1. Title: The Effect of Cigarette Smoke on the Immunological and Metabolic Function of Alveolar Macrophages. Principal Investigator: J.J. Guarneri, Ph.D. - Amount: $29,380.

7/71 - 6/72 - Saint Vincent Hospital Research Foundation. Title: The Influence of Bacterial Species on the Anti-bacterial Activity of Alveolar Macrophages. Principal Investigator: J. J. Guarneri, Ph.D. Amount $ 4,000.

7/71 - 6/75 - The Council for Tobacco Research - U.S.A. Grant Award # 547C. Title: The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Muco-ciliary Function. Principal Investigator: J.J. Guarneri, Ph.D. - Amount: $63,000.

5/74 - 6/75 - Long Island Jewish-Hillside Medical Center #274. Title: Important Determinants of Pulmonary Resistance to Infection, Alcoholic Intoxication. Principal Investigator: J. J. Guarneri, Ph.D. - Amount: $22,413.

PUBLICATIONS: (Papers published or in press)

1. Laurenzi, G.A., Guarneri, J.J., Endriga, R.B. and Carey, J.P.: Clearance of Bacteria by the Lower Respiratory Tract. Science: 142: 1572-1573, 1963.

2. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B. "Important Determinants in Pulmonary Resistance to Bacterial Infections." In the Pathogenisis of Chronic Obstructive Broncho-Pulmonary Disease. In Mitchell R.S.: Progress in Research in Emphysema and Chronic Bronchitis, New York, S. Karger, 1965, p. 45-59.

3. Laurenzi, G.A., Guarneri, J. J. and Endriga, R.B.: Important Determinants in Pulmonary Resistance to Bacterial Infection. Medicinia Thoracalis 22: 48-59, 1965.

4. Laurenzi, G.A., and Guarneri, J.J.: A Study of the Mechanisms of Pulmonary Resistance to Infection: The Relationship of Bacterial Clearance to Ciliary and Alveolar Macrophage Function. Symposium on Structure, Function, and Measurement of Respiratory Cilia. Am. Rev. of Respiratory Dis. 93: 134-141, 1966.

5. Laurenzi, G.A., Yin, S., Collins, R., Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Current Research in Chronic Obstructive Lung Diseease. U.S. Public Health Service Publication No. 1787: 27-40, 1967.

6. Laurenzi, G.A., Yin, S., and Guarneri, J. J.: The Adverse Effect of Oxygen on Tracheal Mucus Flow. New England J. of Med. 279: 333 - 339, 1968.

7. Guarneri, J.J. and Laurenzi, G.A.: The Effect of Alcohol on the Mobilization of Alveolar Macrophages. J. of Lab. and Clinical Med. 72: 40-51, 1968.

8. Combs, T.J., Guarneri, J.J., and Pisano, M.A. The Effect of Sodium Chloride on the Lipid Contents and Fatty Acid Composition of Candida Albicans. Mycologia LX: 1232 - 1239, 1968.

9. Guarneri, J.J.: Clearance of Inhaled Bacteria from the Murine Respiratory Tract. In Developments In Industrial Microbiology. American Institute of Biological Sciences, Washington, D.C., Volume 15, 1974 (In Press).

10. Guarneri, J.J.: Influence of Acute Exposure to Cigarette Smoke on the Alveolar Macrophage System. J. Lab. Clin. Med., 1975. Submitted to CTR for publication support.

ABSTRACTS: (abstracts published and presented or read by title)

1a. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Bacterial Clearance from the Lung of Mice, Fed. Proc.: 22: 255, 1963.

2a. Laurenzi, G.A., Endriga, R.B., Guarneri, J.J. and Carey, J.P.: Important Determinants in Resistance to Pulmonary Infection. J. Clinical Invest. 949: 42, 1963.

3a. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Important Determinants in Pulmonary Resistance to Infection: Proceeding of Seventh Aspen Conference on Bronchitis and Pulmonary Emphysema. p. I-4, 1964.

4a. Laurenzi, G.A., Collins, B.J., Yin S and Guarneri, J.J.: The Adverse Effects of High Oxygen Breathing and Hypoxia. J. Clin Invest. 45: 1035, 1966.

5a. Laurenzi, G.A., Yin, S., Collins, B. J. and Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Proceedings of the Tenth Aspen Conference. p. 100, 1966.

6a. Laurenzi, G.A., Collins, B.J., Yin, S. and Guarneri, J.J.: Adverse Effect of High Oxygen Breathing on Tracheobronchial Mucus Flow. Amer. Rev. Resp. Dis. Vol. 96: 152, 1967.

7a. Guarneri, J.J., Combs, T.J., and Pisano, M.A.: Lipid Components of Candida stellatoide Bacteriol Proc. p. 84, 1967.

8a. Guarneri, J.J.: Lipid Composition of Candida stellatoides. Disertation Abstracts 27: 3614-B, 1967.

9a. Guarneri, J.J. and Laurenzi, G.A.: The Mobilization of Alveolar Macrophages as a Pulmonary Defense Mechanism Against Inhaled Bacteria. Bacteriol Proc. p. 100, 1968.

10a. Laurenzi, G.A., Yin, S., Collins, B.J. and Guarneri, J.J.: Mucus Flow in the Mamallian Trachea. Public Health Service Publication No. 1787, p. 27, 1967.

11a. Combs, T.J., Guarneri, J.J. and Pisano, M.A.: Effect of Growth Conditions on the Fatty Acid Composition of Candida Albicans. The Third Symposium on Yeasts, Delft-Hague, The Netherlands, June 2-7, 1969.

12a. Guarneri, J.J. and Laurenzi, G.A.: The Effect of Cigarette Smoke on Alveolar Macrophage Numbers. The 148th Annual Meeting of the New York City Branch of the American Society for Microbiology, New York, N.Y., Feb. 25, 1971.

13a. Guarneri, J.J. and Laurenzi, G.A.: Influence of Cigarette Smoke on Pulmonary Defense Against Inhaled Bacteria. Alveolar Macrophage Numbers and Viability. The 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., May 26, 1972. (Abstract No. M 170).

14a. Guarneri, J.J. and Sierra, M.F.: Antibacterial Activity of Alveolar Macrophages Against Staphylococcus aureus. The 1974 Annual Meeting of The American Society for Microbiology, New York City Branch, Wagner College, Staten Island, New York, April 15, 1974.

15a. Guarneri, J.J.: Influence of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. The 1974 Annual Meeting of The American Society for Microbiology, New York City Branch, Wagner College, Staten Island, New York, April 15, 1974.

16a. Guarneri, J.J.: Influence of Extended Exposure to Cigarette Smoke on Pulmonary Defense against Inhaled Bacteria. 74th Annual Meeting of The American Society for Microbiology, Chicago, Ill, May 12-17, 1974. (Abstract No. M355).

17a. Guarneri, J.J.: Clearance of Inhaled Bacteria from Murine Respiratory Tract. 25th Annual Meeting of the Society for Industrial Microbiology, Memphis, Tenn., Aug. 11 - 16, 1974, (Abstract).

18a. Guarneri, J.J.: Influence of Acute Exposure to Cigarette Smoke on Pulmonary Defense Mechanisms. 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif. Sept. 11-13, 1974. (Abstract No. 181).

19a. Guarneri, J. and Goldstein, J.: A Study of the In-Vitro Interaction Between Alveolar Macrophages and Staphylococcus aureus. The 1975 Annual Meeting of The New York City Branch of the American Society for Microbiology, Wagner College, Staten Island, New York, January 14, 1975.

PRESENTATIONS: (papers given by invitation and thesis)

1p. Guarneri, J.J.: The Inhibition of Bacteria by Aconitic Acid. Master's Thesis, St. John's University, June 9, 1963.

2p. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Important Determinants in Pulmonary Resistance to Infection: Proceedings of Seventh Aspen Conference on Bronchitis and Pulmonary Emphysema, p. 1-4, 1964.

3p. Laurenzi, G.A., Yin, S., Collins, B.J. and Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Proceedings of the Tenth Aspen Conference, p. 100, 1966.

4p. Guarneri, J.J.: Lipid Composition of Candida stellatoidea. Ph.D. Thesis, St. John's University, June 12, 1966.

Biographical Sketch - Boris A. Shidlovsky, Ph.D.

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TELEPHONE: Home: REDACTED
Office: 212-990-2337

DATE OF BIRTH: REDACTED

MARITAL STATUS:

EDUCATION: R - New York University B.A.
- St. John's University M.S.
- St. John's University Ph.D.

MILITARY SERVICE: 1942 - 1946 U.S. Army Medical Corps. senior non-commissioned officer.

LICENSURE STATUS: Certificate of Qualification for Director of a Clinical Microbiology Laboratory, City of New York, Department of Health.

PROFESSIONAL EXPERIENCE:

1951-1961	Div. of Surg. Res. Lab. Harlem Hospital, New York City, Bacteriologist-in-charge.
1961-1962	Microbiology Department, New York University Dental School, New York, Research Associate.
1962-1963	Misericordia Hospital, New York, Chief Bacteriologist
1963-1966	Morrisania Hospital (Montefiore-Morrisania Hospital Affil.), Chief Bacteriologist
1966-1969	Quinton Research Labs/Merck & Co., Inc. (Senior Research Microbiologist)
1969-1974	Associate Professor at Monmouth College, West Long Branch, New Jersey
1974	Assistant Attending Microbiologist, Long Island Jewish-Hillside Medical Center/ Queens Hospital Center.

MEMBERSHIPS IN PROFESSIONAL SOCIETIES: REDACTED

REDACTED

10035463339

MEMBERSHIPS IN PROFESSIONAL SOCIETIES:

REDACTED

RESEARCH INTERESTS: Antimicrobial agents and host defense mechanisms

13. Publications Pertinent to Material Covered in Grant Proposal*:

1. Guarneri, J. J. and Laurenzi, G.A. The Effect of Cigarette Smoke on Alveolar Macrophage Numbers. The 148th Annual Meeting of the New York City Branch of the American Society for Microbiology, New York City, Feb. 25, 1971.

2. Guarneri, J.J. and Laurenzi, G.A. Influence of Cigarette Smoke on Pulmonary Defense Against Inhaled Bacteria: Alveolar Macrophage Numbers and Viability. The 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., May 26, 1972. (Abstract No. M 170).

3. Guarneri, J.J. and Sierra, M.F. Antibacterial Activity of Alveolar Macrophages Against Staphylococcus aureus. The 1974 Annual Meeting of the American Society for Microbiology, New York City Branch, New York, April 15, 1974.

4. Guarneri, J.J. Influence of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. The 1974 Annual Meeting of the American Society for Microbiology, New York City Branch, New York, April 15, 1974.

5. Guarneri, J. J. Influence of Extended Exposure to Cigarette Smoke on Pulmonary Defense against Inhaled Bacteria. 74th Annual Meeting of the American Society for Microbiology, Chicago, Ill. May 12-17, 1974. (Abstract No. M 355).

6. Guarneri, J.J. Clearance of Inhaled Bacteria from the Murine Respirator Tract. The 25th Annual Meeting of the Society for Industrial Microbiology, Memphis, Tenn., Aug. 11-16, 1974.

7. Guarneri, J.J. Influence of Acute Exposure to Cigarette Smoke on Pulmonary Defense Mechanisms. The 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., Sept. 11-13, 1974. (Abstract No. 181).

8. Guarneri, J.J. Clearance of Inhaled Bacteria from the Murine Respirator Tract. In: Developments In Industrial Microbiology. American Institute of Biological Sciences, Washington, D.C., Volume 16, 1975 (In Press).

9. Guarneri, J.J. Influence of Acute Exposure to Cigarette Smoke on the Alveolar Macrophage System. Journal of Lab. and Clin. Med., 1975. Submitted to CTR for publication support.

10. Guarneri, J. J. and Goldstein, J. .A study of the In Vitro Interaction Between Alveolar Macrophages and Staphylococcus aureus. The 1975 Annual Meeting of the American Society for Microbiology, New York City Branch, New York. Jan. 14, 1975.

11. Guarneri, J.J. Influence of In Vivo Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. Submitted for presentation at: The 75th Annual Meeting of the American Society for Microbiology, New York, 1975.

12. Guarneri, J.J., Goldstein, J. and Shidlovsky, B. Effect of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. Submitted for presentation at: The 75th Annual Meeting of The American Society for Microbiology, New York, 1975.

* See Curriculum Vitae page 9 for a complete list of publications.
See Addendum II for copies of publications pertinent to material covered in grant proposal.

13. Publications Pertinent to Material Covered in Grant Proposal:

13. Guarneri,J.J. Influence of Acute Exposure to Cigarette Smoke on the Clearance of Bacteria by the Murine Respiratory Tract. (In Preparation).

14. Guarneri,J.J. Influence of Extended Exposure to Cigarette Smoke on the Clearance of Bacteria by the Murine Respiratory Tract. (In Preparation).

Addendum II. Pertinent Personal Publications

Table of Contents

1. Laurenzi, G.A., Guarneri, J.J., Endriga, R.B. and Carey, J.P.: Clearance of Bacteria by the Lower Respiratory Tract. Science: 142: 1572-1573, 1963.

2. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Bacterial Clearance from the Lung of Mice, Fed. Proc.: 22: 255, 1963.

3. Laurenzi, G.A., Endriga, R.B., Guarneri, J.J. and Carey, J.P.: Important Determinants in Resistance to Pulmonary Infection. J. Clinical Invest. 949: 42, 1963.

4. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Important Determinants in Pulmonary Resistance to Infection: Proceeding of Seventh Aspen Conference on Bronchitis and Pulmonary Emphysema. p. 1-4, 1964. (Copy not available).

5. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B. "Important Determinants in Pulmonary Resistance to Bacterial Infections". In the Pathogenisis of Chronic Obstructive Broncho-Pulmonary Disease. In Mitchell, R.S.: Progress in Research in Emphysema and Chronic Bronchitis, New York, S. Karger, 1965, p. 45-59. (Copy not available).

6. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.R.: Important Determinants in Pulmonary Resistance to Bacterial Infection. Medicinia Thoracalis 22: 48-59, 1965.

7. Laurenzi, G.A., Collins, B.J., Yin S. and Guarneri, J.J.: The Adverse Effects of High Oxygen Breathing and Hypoxia. J. Clin Invest. 45: 1035, 1966.

8. Laurenzi, G.A., and Guarneri, J.J.: A Study of the Mechanisms of Pulmonary Resistance to Infection: The Relationship of Bacterial Clearance to Ciliary and Alveolar Macrophage Function. Symposium on Structure, Function, and Measurement of Respiratory Cilia. Am. Rev. of Respiratory Dis. 93: 134-141, 1966.

9. Laurenzi, G.A., Yin, S., Collins, B.J. and Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Proceedings of the Tenth Aspen Conference. p. 100, 1966. (Copy not available).

10. Laurenzi, G.A., Collins, B.J., Yin, S. and Guarneri, J.J.: Adverse Effect of High Oxygen Breathing on Tracheobronchial Mucus Flow. Amer. Rev. Resp. Dis. Vol. 96: 152, 1967.

11. Laurenzi, G.A., Yin, S., Collins, R., Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Current Research in Chronic Obstructive Lung Disease. U.S. Public Health Service Publication No. 1787: 27-40, 1967.

12. Laurenzi, G.A., Yin, S., Collins, B.J. and Guarneri, J.J.: Mucus Flow in the Mamallian Trachea. Public Health Service Publication No. 1787, p. 27, 1967. (Abstract). (Copy not available).

13. Guarneri, J.J. and Laurenzi, G.A.: The Mobilization of Alveolar Macrophages as a Pulmonary Defense Mechanism Against Inhaled Bacteria. Bacteriol Proc. p. 100, 1968.

14. Laurenzi, G.A., Yin, S., and Guarneri, J.J.: The Adverse Effect of Oxygen on Tracheal Mucus Flow. New England J. of Med. 279: 333 - 339, 1968.

Addendum II. Pertinent Personal Publications (cont'd.)

15. Guarneri, J.J. and Laurenzi, G.A.: The Effect of Alcohol on the Mobilization of Alveolar Macrophages. J. of Lab. and Clinical Med. 72: 40-51, 1968.

16. Guarneri, J.J. and Laurenzi, G.A.: The Effect of Cigarette Smoke on Alveolar Macrophage Numbers. The 148th Annual Meeting of the New York City Branch of the American Society for Microbiology, New York, N.Y., Feb. 25, 1971.

17. Guarneri, J.J. and Laurenzi, G.A.: Influence of Cigarette Smoke on Pulmonary Defense Against Inhaled Bacteria. Alveolar Macrophage Numbers and Viability. The 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., May 26, 1972. (Abstract No. M 170).

18. Guarneri, J.J. and Sierra, M.F.: Antibacterial Activity of Alveolar Macrophages Against Staphylococcus aureus. The 1974 Annual Meeting of The American Society for Microbiology, New York City Branch, Wagner College, Staten Island, New York, April 15, 1974.

19. Guarneri, J.J.: Influence of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. The 1974 Annual Meeting of The American Society for Microbiology, New York City Branch, Wagner College, Staten Island, New York, April 15, 1974.

20. Guarneri, J.J.: Influence of Extended Exposure to Cigarette Smoke on Pulmonary Defense against Inhaled Bacteria. 74th Annual Meeting of The American Society for Microbiology, Chicago, Ill. May 12-17, 1974. (Abstract No. M 355)

21. Guarneri, J.J.: Clearance of Inhaled Bacteria from Murine Respiratory Tract. 25th Annual Meeting of the Society for Industrial Microbiology, Memphis, Tenn., Aug. 11-16, 1974. (Abstract)

22. Guarneri, J.J.: Clearance of Inhaled Bacteria from the Murine Respiratory Tract. In Developments in Industrial Microbiology. American Institute of Biological Sciences, Washington, D.C., Volume 16, 1974 (In Press).

23. Guarneri, J.J.: Influence of Acute Exposure to Cigarette Smoke on Pulmonary Defense Mechanisms. 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif. Sept. 11-13, 1974. (Abstract No. 181).

24. Guarneri, J. and Goldstein, J.: A Study of the In-Vitro Interaction Between Alveolar Macrophages and Staphylococcus aureus. The 1975 Annual Meeting of The New York City Branch of the American Society for Microbiology, Wagner College, Staten Island, New York, January 14, 1975.

25. Guarneri, J.J.: Influence of Acute Exposure to Cigarette Smoke on the Alveolar Macrophage System. J. Lab. Clin. Med., 1975. Submitted to CTR for publication support.

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27. Guarneri, J.J., Goldstein, J. and Shidlovsky, B.: Effect of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. Submitted for presentation at: The 75th Annual Meeting of the American Society for Microbiology, New York, 1975.